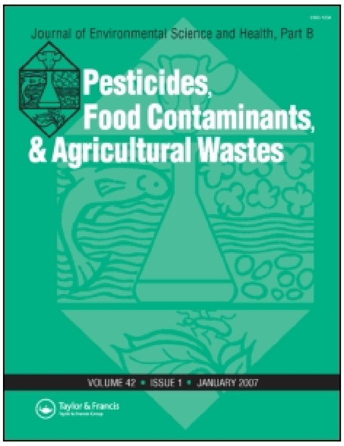

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Level and fate of chlorpropham in potatoes during storage and processing

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Chlorpropham (isopropyl 3-chlorocarbanilate) is a pesticide used to control sprouting of potatoes during long-term storage. The objective of the present study was to establish the total chlorpropham residue balance (residues in unwashed and washed whole tubers, peeled tubers, peels, boiled and pureed tubers, and washing and cooking waters) for two potato varieties after uniform application as a function of storage time under different typical storage conditions (in a basement, storehouse, and refrigerator) in Lithuania. Chlorpropham concentration on washed and unwashed tubers decreased from ~15 mg kg^{-1} after storage for 28 d to ~9 mg kg^{-1} after storage for 85 d. Peel concentrations decreased from ~50 mg kg^{-1} at 5 d after treatment to ~20 mg kg^{-1} at 85 d after treatment. The average concentration in the two varieties of peeled tubers in the three storage facilities was 1 mg kg^{-1}. Chlorpropham concentrations in the wash water decreased from 3.5 to 1.0 mg L^{-1} when the storage time increased from 28 to 85 d. The cooking water concentration similarly decreased, from >0.2 mg L^{-1} at 28 d after treatment to >0.1 mg L^{-1} at 85 d after treatment. The results of this study show that chlorpropham concentrations in tubers under these typical conditions were below the recently revised levels that are acceptable for residues in whole potatoes (30 mg kg^{-1}) and peels (40 mg kg^{-1}). Also, despite fluctuating conditions during storage, chlorpropham treated tubers did not sprout, as compared to untreated tubers, which sprouted.

Keywords: Chlorpropham; potatoes; dissipation; storage conditions.

Introduction

During the past 40 years there has been continued concern about the safety of the food supply. The presence of pesticides in particular has been regarded as a potential risk to human health. Residues of pesticides applied pre-harvest to crops to control weeds, insects, and diseases have been found in a variety of fruits and vegetables, including potatoes, in different countries. For instance, residues of aldicarb ([(2-methyl-2-methylsulfanyl-propylidene)amino] *N*-methylcarbamate) (UK),[1] metalaxyl (methyl 2-[(2,6-dimethylphenyl)-(2-methoxyacetyl)amino]propanoate) (Spain),[2] dithiocarbamates (Slovenia),[3] endosulfan, DDT, imazalil (1-[2-(2,4-dichlorophenyl)-2-prop-2-enoxy-ethyl]imidazole), and procymidone (3-(3,5-dichlorophenyl)-1,5-dimethyl-3-azabicyclo[3.1.0]hexane-2,4-dione) (Denmark)[4] and malathion, hexachlorbenzene (HCB), lindane, and p,p-1, 1,-dichlor-2-2bis(p-chlorophenyl)ethane (DDD) (Egypt)[5] have been reported in potatoes. Also, analysis of pesticide residues in organic food samples in Italy has even shown the presence of four different pesticides in potatoes, one of which was at levels above the maximum residue level (MRL) permitted.[6]

In addition to pesticides being applied pre-harvest to potatoes, pesticides are also applied post-harvest to control mold (thiabendazole) (2-(1,3-thiazol-4-yl)-1*H*-benzoimidazole) and insects [i.e. pyrethrins and diclorvos (1,1-dichloro-2-dimethoxyphosphoryloxy-ethene)] and to suppress sprouting of the potatoes (i.e. chlorpropham) during storage. These chemicals have been found in processed food made from the treated potatoes. For instance, thiabendazole has been detected in potato crisps[7] and extruded potato peels,[8] while pyrethrins have been detected in dried starch.[9] Chlorpropham has been detected in potato crisps,[7] potato chips,[10] french fries,[11] and extruded potato peels.[8]

To be effective, post-harvest pesticides have to be applied at sufficiently high concentrations to persist throughout the storage period, which in turn may require multiple applications during the storage period. Although it appears that chlorpropham dissipates with time to concentration levels below acceptable tolerance levels, it is difficult to

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calculate dissipation rates or compare data between studies to characterize factors controlling dissipation. For instance, initial application rates may vary depending on the storage temperature, the length of intended storage, or method of application. Chlorpropham has been applied as different formulations and application methods, i.e. gas, fog, aerosol, dust, or granular,[12–14] with retreatment often necessary to extend sprout inhibition during storage.[14–16] These methods often result in a lack of uniform chlorpropham concentration on the tubers. For instance, after aerosol or direct spray treatment, chlorpropham concentrations significantly vary between potatoes at the top, middle, and bottom of the bulk pile of potatoes.[14–15] Potatoes stored in piles and treated by aerosol had uneven distribution of chlorpropham, presumably because of differential airflow within the piles.[13] Also, in a study of treated unwashed potatoes, up to 45% of applied chlorpropham was found in soil adhering to the tubers, which could be removed with the soil during washing.[17]

Until effective alternative natural products, such as carvone or menthone[18] (for a review see Kleinkopf et al. [19]) are commercially developed as effective sprout suppressants, pesticides such as chlorpropham will be continue to be used, and information on residue levels is needed to maintain consumer health and safety. Potato cultivars differ in chlorpropham residue concentrations necessary for sprout control, which is also influenced by storage conditions and temperatures.[19] The objectives of the present study were to: 1) establish the total chlorpropham residue balance (residues in raw materials, intermediate products, final products, solid and liquid wastes) in potatoes after uniform application as a function of storage time under different typical storage conditions in Lithuania; and 2) determine whether the chlorpropham concentrations in tubers under these typical conditions were below the recently revised levels that are acceptable for residues in whole potatoes (30 mg kg^{-1}) and peels (40 mg kg^{-1}).[20]

This research was part of a larger study that examined the fate of selected pesticides (applied either pre- or post-harvest) during processing procedures of commodities with great economic importance for European countries. This study had a special emphasis on products used to prepare baby foods such as peaches, apples, strawberries, potatoes and wheat. The data can then be used to contribute to refinement of risk assessment and risk management of the pesticide residues and estimation of their possible adverse effects on consumers and especially vulnerable groups such as infants and children.

Materials and methods

Treatment and sampling

Lithuanian breeding potato cultivars, Goda and Mirta, were treated with the sprouting inhibitor chlorpropham following 6-mo post-harvest storage at ∼3°C. Many early harvested cultivars are successfully stored 6 to 10 months without chlorpropham treatment[19], after which they must be treated for longer storage. For treatment, 100 unwashed tubers of about 6–10 cm diameter in size were dipped in an aqueous emulsion of 1% chlorpropham for 5 min. In Lithuania, the labeled formulated compounds are Luxan Grow Stop Basis 300 g a.i./L applied as a spray and Neo Stop L 500 HN 500 g a.i./L applied as a fumigant, both of which are currently on the market.

After air drying for 20 min, the tubers were placed in cardboard boxes in layers and the boxes wrapped with the plastic. Control tubers were dipped in water only. The tubers were then stored in the dark in three different facilities at three different temperatures: 5°C in a refrigerator; 12°C in a basement; and at 4°C in a commercial storehouse. However, these temperatures were not uniform during the incubation. In the basement, the temperature varied from 12° to 17°C, while in the storehouse the temperature increased from 4° to 12°C by the end of the experiment.

At each sampling time, 5, 28, 56, and 84 d after treatment, the plastic was removed and 25 tubers were randomly selected. The sampling at 56 d corresponds to the minimum recommended period between application and processing for consumption. The sampled tubers were divided into sub-samples to analyze residues in unwashed and washed whole potatoes, washed peeled raw and boiled pureed potatoes, and potato peels.

At each sampling time, 6–7 unwashed tubers and 6–7 tubers washed in a gentle stream of tap water (∼300 mL), were diced, thoroughly mixed, and triplicate 25-g subsamples removed for analysis. The remaining 12-13 tubers were washed with a gentle stream of tap water (∼700 mL), which was saved for analysis. These washed tubers were peeled with an ordinary kitchen knife (peel thickness ∼ 2mm). Triplicate 25-g subsamples of peels were subsequently analyzed. Half of the peeled tubers were diced, thoroughly mixed, and triplicate 25-g subsamples removed for analysis. The remaining tubers were boiled in ∼700 mL water for ∼25 min. The boiled tubers were removed from the water, pureed, and triplicate 25-g subsamples removed for analysis.

The replicate 25-g subsamples of whole potato (washed and unwashed), peeled potato, potato peels, and boiled pureed potatoes were homogenized for 2–3 min with 75 mL of dichloromethane (DCM) / acetonitrile (ACN)(70:30 v:v). The mixture was centrifuged at 3000 rpm for 15 min and the supernatant organic phase was removed for high performance liquid chromatography (HPLC) analysis. A 1 mL-aliquot was placed into HPLC vials for the analyses.

For analysis of wash and cooking waters, triplicate 100-mL aliquots of water were shaken 3 min with 75 mL (25 mL x 3 times) of dichloromethane (DCM) / acetonitrile (ACN)(70:30 v:v). The organic phases from the three extractions were combined and 1-mL aliquots were placed into HPLC vial for analyses.

Analysis

Chlorpropham residues were analyzed by high performance liquid chromatography (HPLC), using a Waters 2690 HPLC high performance liquid chromatograph equipped with a photodiode array detector set to monitor 240 nm wavelength. Separation of the chlorpropham was done using a Waters Nova-Pak C_{18} 5 μm column (150 mm × 3.9 mm i.d.) and mobile phase, which was a gradient of methanol and 20 mM formic acid, starting at 50% methanol changing linearly to 95% methanol at minute 10 and back to 50% methanol at minute 13. At a mobile phase flow rate of 0.75 mL min^{-1}, the retention time was 7.6 min. The injection volume was 20 μL.

Analytical standard of chlorpropham (94% grade) (Kemira, Lithuania) was used to prepare 100 mL of 100 μg mL^{-1} concentration of chlorpropham in methanol, from which working standard solutions were prepared. The detector response was linear over the concentrations analyzed, 0.05 to 10.0 μg mL^{-1}. The concentration of the 1% chlorpropham water emulsion solution used to treat the potatoes was confirmed by HPLC after dilution to the appropriate concentration range for analysis. Chlorpropham tuber concentrations are expressed on a wet-weight basis.

Results and discussion

Untreated and treated tubers were stored under conditions typically found in countries such as Lithuania, such as basements, storehouses, and refrigerators. Untreated potatoes had started to sprout after 28 d in the basement and sprouts were 1.0–1.5 cm in length (data not shown), whereas untreated potatoes in store-house and refrigerator had no sprouts. At 56 d in basement, the sprouts of untreated potatoes were 15–17 cm length, while those in the storehouse were 4–5 cm in length, and those in the refrigerator had no sprouts. In contrast, despite fluctuating temperatures and humidities during storage under the three methods, chlorpropham-treated tubers did not have sprouts 5 mo after application. Chlorpropham may not be as effective under fluctuating conditions as potatoes stored under fluctuating temperatures and humidities may physiologically age faster than those stored under constant conditions.[19] However, the treatment with chlorproham resulted in sufficient chemical on the tubers to inhibit sprouting throughout the duration of the storage, regardless of storage method.

To inhibit sprouting, a wide range of concentrations of chlorpropham on potatoes have been reported. For instance, no sprouts were observed on tubers stored 75 d if they had a chlorpropham concentration $>\sim 2$ mg kg^{-1}.[21] In contrast, after chlorpropham application in January, tubers would be sprouting in May if they had concentrations <20 mg kg^{-1} in March.[14] Neither of the varieties of tubers used in our study, washed or unwashed, had chlorpropham concentrations above the recently revised tolerance level of 30 mg kg^{-1} at 28 d after treatment (Fig. 1 a, b). In 2002, the tolerance level for whole potatoes was lowered from 50 mg kg^{-1} combined residues of chlorpropham and its 1-hydroxy-2-propyl-3′-chlorocarbanilate metabolite to 30 mg kg^{-1} chlorpropham alone.[20]

Washing removed some chlorpropham from the tubers, as evidenced by the chlorpropham concentration in wash water, which decreased from $\sim$3.5 to 1.0 mg L^{-1} with storage time (Fig. 2). However, the total amount removed was relatively small; in general, there were no significant differences in chlorpropham concentrations between unwashed and washed tubers (Fig. 1a, b). In contrast, other washing studies showed that significant amounts of chlorpropham could be removed from the tubers. For instance, brief, hand washing of tubers in a stream of cold water decreased the chlorpropham concentration 24%, from 3.8 to 2.8 mg kg^{-1} for tubers treated with a dust powder and stored for 24 d.[11] A more exhaustive water washing removed 88% of chlorpropham on tubers treated with an emulsified solution and stored 72 d; the concentration decreased from 1.6 to 0.2 mg kg^{-1}.[9] The differences in removal may be due to differences in rigor of washing, chlorpropham application methods, or differences in varieties used in the studies.

The highest chlorpropham concentration, 25.2 mg kg^{-1}, observed for tubers stored a minimum 28 d after treatment was in the Goda tubers stored in a refrigerator. Chlorpropham concentrations in washed and unwashed Goda tubers stored under the three conditions slowly decreased with storage time (Fig. 1a). The mean concentration for the three storage methods for Goda tubers decreased from 15.5 mg kg^{-1}at Day 28 to 10.2 mg kg^{-1}at Day 85. Concentrations in Mirta tubers decreased from 15.1 to 8.6 mg kg^{-1}during the same time period (Fig. 1b). These concentrations are slightly greater that those previously reported. The amounts of chlorpropham in washed potatoes remaining after 30 d of storage at 5 to 10 °C range from 0[13] to 3[11] mg kg^{-1}when applied as a powder; and 1[9] to 7 [21] mg kg^{-1} when applied as an aerosol or spray. However, the presently reported residue levels are close to the MRL of 5 to 10 mg kg^{-1}envisioned by European Union (EU) member countries.[19]

In view of the difficulties in calculating dissipation rates and of comparing data between studies on characterization of factors controlling dissipation, such as variability in application of powders and aerosols, and chlorpropham sorption to soil adhering to the tubers, in our study chlorpropham dissipation was determined from concentrations in peels of washed tubers, which had been treated by dipping in a solution of chlorpropham. At 5 d after treatment, except for Goda tubers in the store house, peel concentrations were above the recently adopted acceptable tolerance limit of 40 mg kg^{-1}for wet peels.[20] After a 28-d storage period of and longer however, all tuber peel concentrations were $<$40 mg kg^{-1}; at Day 85, peel concentrations were 18–25 mg kg^{-1}. Storage conditions did not influence amount of chlorpropham remaining in the peel. The mean

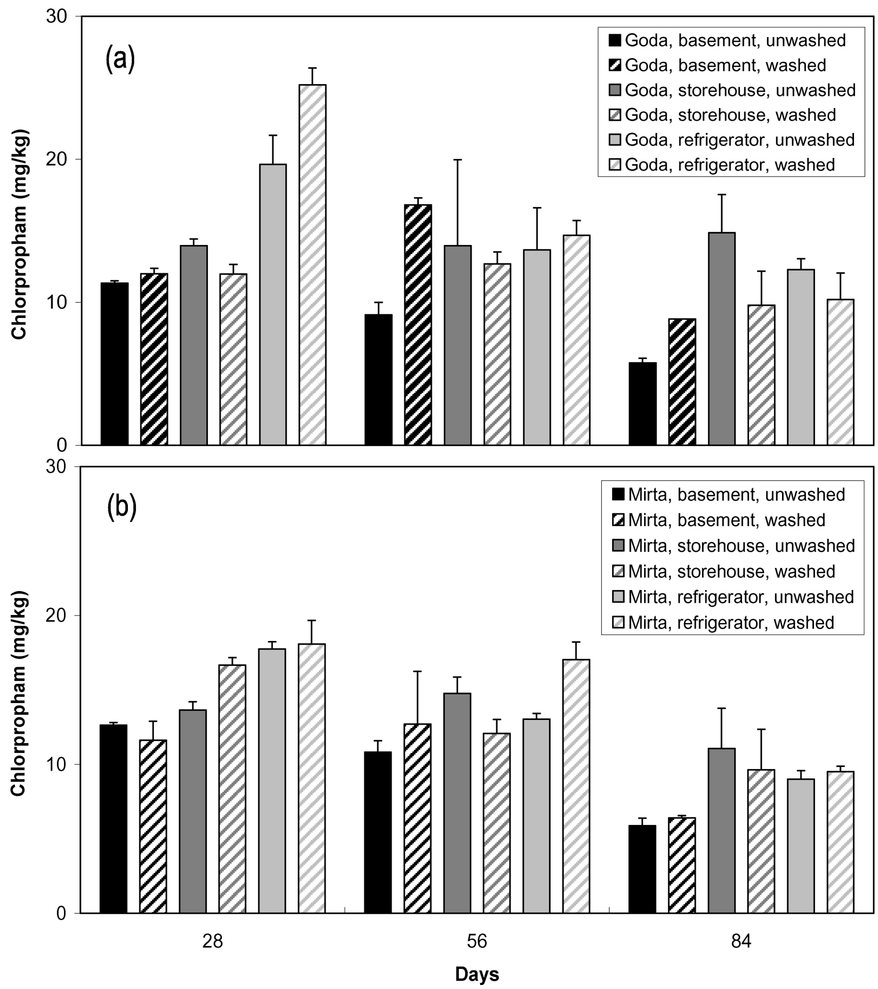

Fig. 1. Chlorpropham concentrations in washed and unwashed whole Goda and Mirta tubers stored up to 85 d in a basement, storehouse, and refrigerator. Bars represent standard errors of the means.

concentration in Goda peels for tubers stored in the three locations decreased from 50.1 mg kg^{-1} at 5 d after treatment to 20.9 mg kg^{-1} at 85 d after treatment; 58% dissipated in 80 d (Fig. 3). Mean Goda peel residue data were not significantly different from the mean concentrations in washed Mirta potato peels at the same times, 46.6 mg kg^{-1} at 5 d after treatment and 21.9 mg kg^{-1} at 85 d after treatment; 53% dissipated in 80 d (Fig. 3). Although it has also been suggested that differences in chlorpropham concentrations during storage may be due to differences in morphology of different varieties[22] it was not the case with Goda and Mirta varieties. Although chlorpropham concentrations in peels have been reported to be extremely variable, i.e. 15 to 293 mg kg^{-1},[14] the values reported in the current study are similar to those reported in other studies (33–34 mg kg^{-1}).[8,11]

After washing and peeling, some chlorpropham remained in the raw potato, regardless of storage method. At 56 d after treatment, the mean concentrations in Goda and Mirta tubers in the three storage facilities were 0.70 and 1.47 mg kg^{-1}, respectively (Fig. 4). The concentrations did not significantly change through the remaining storage period. Cooking appeared to have removed some of the chlorpropham from the potato, either through desorption from the potato or degradation during the boiling process. The average concentrations in pureed potatoes at 56 d after treatment were 0.51 and 0.37 in Goda and Mirta potatoes, respectively, significantly lower than the corresponding raw potato (Fig. 4). Although we did not analyze for chlorpropham metabolites, the chlorpropham concentration in the cooking water decreased from 0.19 mg L^{-1} at 28 d to 0.05 mg L^{-1} at 85 d after treatment for Goda tubers and from 0.10 to 0.07 mg L^{-1}for Mirta tubers (Fig. 2).

This study was conducted using storage conditions typically found in Lithuania and surrounding countries. In order to obtain a uniform chlorpropham application for the comparison of dissipation under different storage conditions, the tubers were dipped in chlorpropham solution as opposed to being treated with a powder, dust, or aerosol formulation. The tubers were then initially stored at three

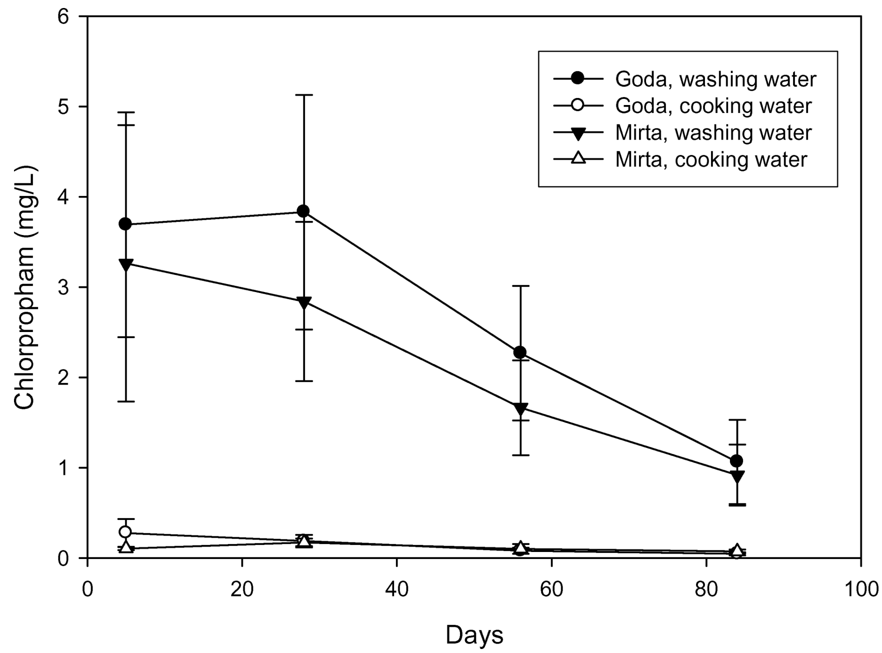

Fig. 2. Mean chlorpropham concentrations in washing and cooking water from Goda and Mirta tubers stored up to 85 d in a basement, storehouse, and refrigerator. Bars represent standard errors of the means.

different temperatures, 12°C in the basement, 4°C in the storehouse; and 5°C in the refrigerator. However the temperatures were not uniform throughout the experiment. The only constant temperature was in the refrigerator, whereas the temperature in the basement increased to 17°C by the end of the experiment in the spring, and the storehouse temperature increased to 12°C. The results of this study show that chlorpropham concentrations in tubers under these typical conditions were below the recently revised levels that are acceptable for residues in both whole potatoes and peels. Also, in spite of fluctuating conditions during the varied storage methods, chlorpropham-treated tubers did not sprout.

However, the fact remains that chlorpropham residues will be detected on potatoes when used for sprout control. While peeling would remove the majority of the chemical, it would also remove nutrients from the potato. It appears that until alternative sprout control methods become commercially available, emphasis should be placed on cleaning of the tuber surface to remove chlorpropham residues if

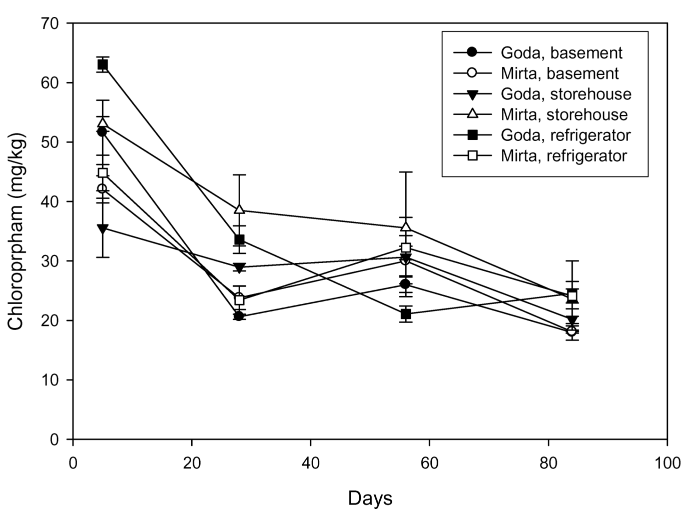

Fig. 3. Chlorpropham concentrations in Goda and Mirta tuber peels stored up to 85 d in a basement, storehouse, and refrigerator. Bars represent standard errors of the means.

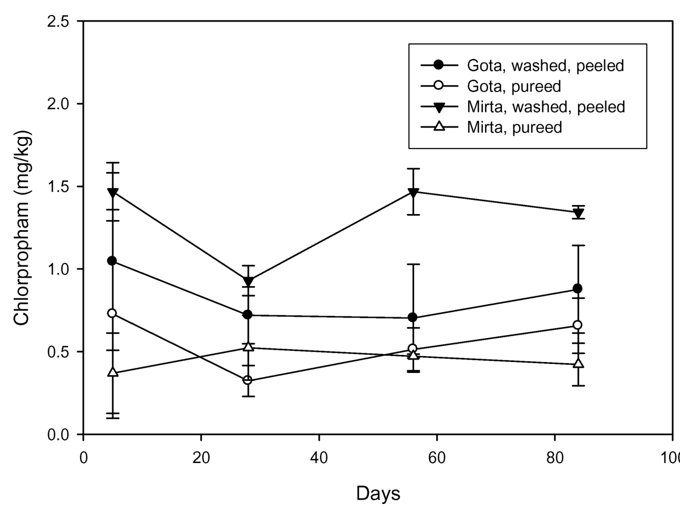

Fig. 4. Mean chlorpropham concentrations in washed, peeled and pureed Goda and Mirta tubers stored up to 85 d in a basement, storehouse, and refrigerator. Bars represent standard errors of the means.

treated with dust powders, aerosols, or emulsifiable solutions, as has been shown in previously discussed literature. Otherwise, until there has been refinement of risk assessment and risk management of the pesticide residues and estimation of their possible adverse effects on vulnerable groups such as infants and children, the emphasis should be placed on use of cultivars that have been shown to be able to be stored successfully up to 6 months and longer without treatment.

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